Claims

1. (original) A kit of reagents for nucleic acid amplification comprising:

a single-stranded external control polynucleotide, a forward primer, a reverse primer, a polymerase, a detectable probe, and one or more nucleotide 5'-triphosphates;

wherein the forward primer and the detectable probe are separated by 0 to 5 nucleotides when hybridized to the external control polynucleotide, or its complement, and the reverse primer and the detectable probe are separated by 0 to 5 nucleotides when hybridized to the external control polynucleotide, or its complement.

2. (original) The kit of claim 1 wherein the forward primer and reverse primer are each 10 to 40 nucleotides in length.

3. (original) The kit of claim 1 wherein the single-stranded external control polynucleotide is 30 to 110 nucleotides in length.

4. (original) The kit of claim 1 wherein the single-stranded external control polynucleotide is 50 to 70 nucleotides in length.

5. (original) The kit of claim 1 wherein the external control polynucleotide, or its complement, forms single-stranded overhangs consisting of 1 to about 10 nucleotides when hybridized to the forward primer or to the reverse primer.

6. (original) The kit of claim 1 wherein said polymerase is a thermostable polymerase with 5' nuclease activity.

7. (original) The kit of claim 1 wherein the detectable probe comprises a fluorescent dye.

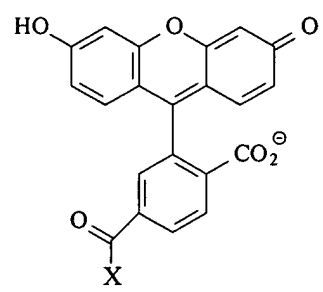
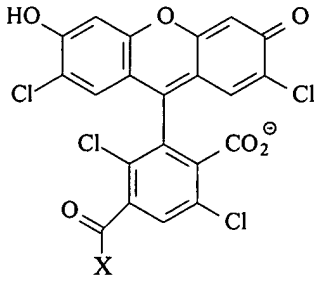
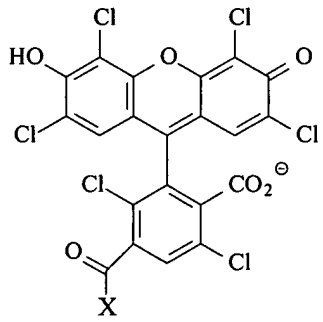
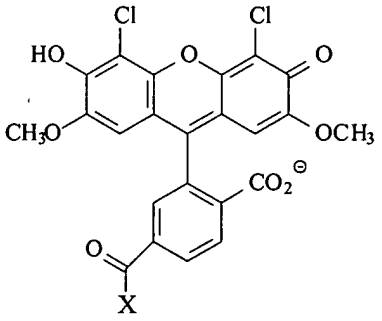
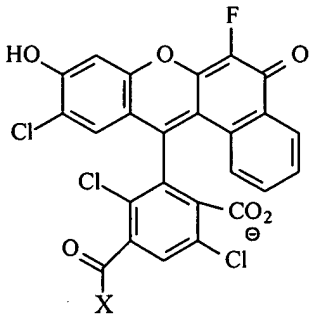
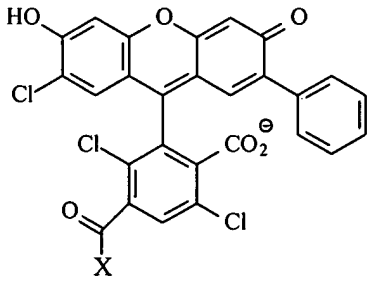
8. (original) The kit of claim 1 wherein the detectable probe is a self-quenching fluorescence probe comprising a reporter dye and a quencher.

9. (original) The kit of claim 8 wherein the self-quenching fluorescence probe is 10 to 40 nucleotides in length.

10. (original) The kit of claim 8 wherein said reporter dye is a xanthene dye.

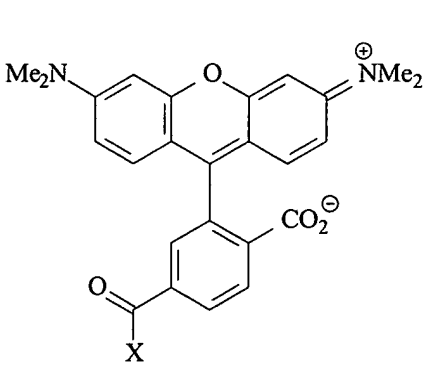
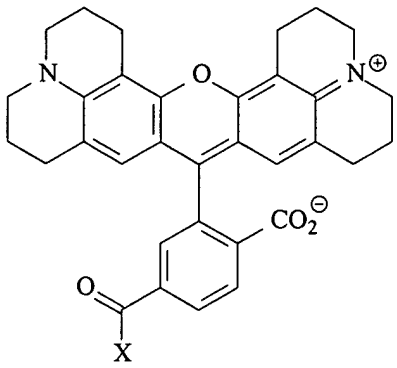
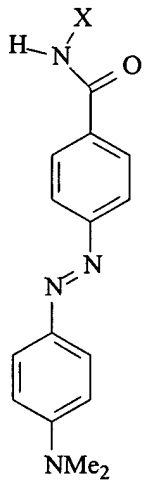
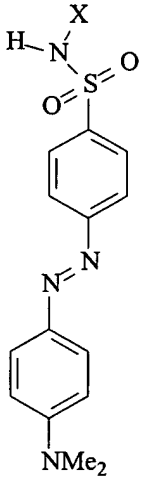
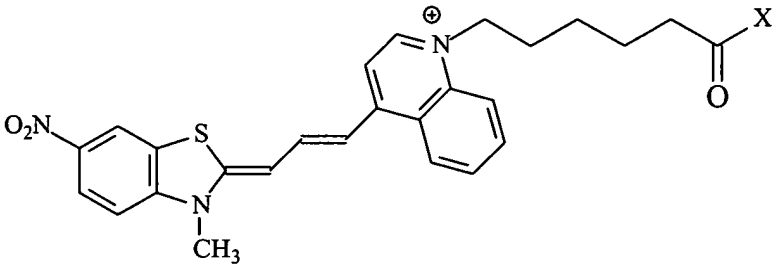
11. (original) The kit of claim 10 wherein said xanthene dye is a fluorescein dye.

12. (original) The kit of claim 11 wherein said fluorescein dye is selected from the group consisting of:

where X is an attachment site to the probe.

13. (original) The kit of claim 8 wherein said quencher is selected from the group consisting of:

where X is an attachment site to the probe.

14. (original) The kit of claim 8 wherein said reporter dye is separated from said quencher by at least 12 nucleotides.

15. (original) The kit of claim 8 wherein said reporter dye is attached at a 5' terminus or a 3' terminus of the self-quenching fluorescence probe.

16. (original) The kit of claim 8 wherein said quencher is attached at a 5' terminus or a 3' terminus of the self-quenching fluorescence probe.

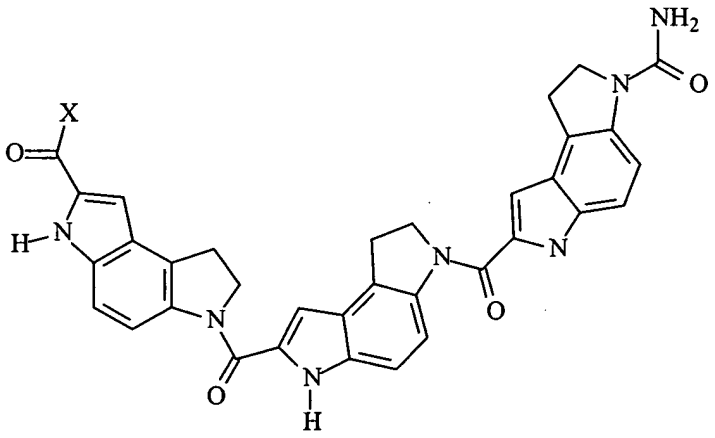
17. (original) The kit of claim 8 wherein said quencher is non-fluorescent.

18. (original) The kit of claim 1 wherein the detectable probe is labelled with a minor groove binder.

19. (original) The kit of claim 8 wherein the self-quenching fluorescence probe is labelled with a minor groove binder.

20. (original) The kit of claim 8 wherein the self-quenching fluorescence probe is labelled with a minor groove binder at a 3' terminus nucleotide.

21. (original) The kit of claim 19 wherein the minor groove binder has the structure:



where X is an attachment site to the probe.

22. (original) The kit of claim 1 where one or more nucleotide 5'-triphosphates comprises a fluorescent dye, a quencher, biotin, or a minor groove binder.

23. (original) The kit of claim 8 further comprising a second self-quenching fluorescence probe comprising a reporter dye and a quencher wherein the sequences of the first self-quenching fluorescence probe and second self-quenching fluorescence probe differ by a single nucleotide.

24. (original) The kit of claim 1 wherein the concentration of the forward primer and the concentration of the reverse primer is each about 10 to 100 μM, the concentration of each nucleotide 5'-triphosphate is about 100 to 1000 μM, and the concentration of the self-quenching fluorescence probe is about 1 to 100 μM.

25. (original) The kit of claim 1 further comprising a second single-stranded external control polynucleotide wherein the forward primer and the detectable probe are separated by 0 to 5 nucleotides when hybridized to the second external control polynucleotide, or its complement, and the reverse primer and the detectable probe are separated by 0 to 5 nucleotides when hybridized to the external control polynucleotide, or its complement; and

wherein the sequence complementary to the detectable probe of the first single-stranded external control polynucleotide differs from the sequence complementary to the detectable probe of the second single-stranded external control polynucleotide by one or more nucleotides, nucleotide insertions, or nucleotide deletions.

26. (previously presented) The kit of claim 1 where the reagents are delivered by robotic means to one or more vessels.

27. (original) The kit of claim 26 where the reagents are spotted on an absorbent or porous material.

28. (original) The kit of claim 26 where the reagents are spotted on a non-absorbent and planar surface.

29. (original) The kit of claim 26 wherein the reagents are located in an array configuration having 6 to 1536 reaction sites.

30. (original) The kit of claim 29 wherein the reagents are located in a microwell tray having 96 to 384 wells.

31. (original) The kit of claim 30 wherein each well has a volume from 1 to 500 μl.